

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:)	Docket No.:	4258-117
)		
Applicants:)	Conf. No.:	6063
ALONSO FERNANDEZ,)		
Maria Jose, et al.)		
)		
Application No.:)	Art Unit:	1618
10/563,031)		
Date Filed:)	Examiner:	Nissa M. Westerberg
December 30, 2005)		
)		
Title:)	Customer No.:	23448
NANOPARTICLES OF)		
POLYOXYETHYLENATED)		
DERIVATIVES)		

DECLARATION OF ANA ISABEL VILA PENA AND BALBINA FERNANDEZ
MARTINEZ UNDER 37 CFR 1.132 IN U.S. PATENT APPLICATION NO. 10/563,031

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, ANNA ISABEL VILA PENA and BALBINA FERNANDEZ MARTINEZ, hereby declare:

1. THAT we are employees of ADVANCED IN VITRO CELL TECHNOLOGIES, S.L., of Barcelona, Spain (hereafter referred to as "AIVCT"), having degrees in chemistry, and working in the laboratories of such company.
2. THAT we have been requested to conduct experimental work in support of United States Patent Application No. 10/563,031 filed December 30, 2005 in the United States Patent and Trademark Office for "NANOPARTICLES OF POLYOXYETHYLENATED

DERIVATIVES" (hereafter referred to as the "Application"), and assigned to AIVCT, with respect to process technology disclosed in U.S. Patent 5,962,566 issued October 5, 1999 to Christian Grandfils, et al. ("Grandfils").

3. THAT Grandfils discloses a method of making nanoparticles for drug delivery, which requires a blend of an aliphatic polyester polymer and a polyether, a lipophilic or polypeptide drug, and a cholesterol compound as a biocompatible surfactant.
4. THAT we have empirically evaluated the process disclosed in Grandfils of making nanoparticles for drug delivery utilizing a blend of an aliphatic polyester polymer and a polyether, a lipophilic or polypeptide drug, and cholesterol, against a corresponding process in which cholesterol is omitted.
5. THAT in such empirical evaluation of the process disclosed in Grandfils, we prepared particles using a process including cholesterol as described in Grandfils ("Example A") and particles using a corresponding process without cholesterol ("Example B").
6. THAT Example A was carried out according to the following procedure:

Example A - Preparation of Nanoparticles from a Blend With Cholesterol

A blend of polymers was prepared by dissolution of PLGA 503 (22.5 mg) and Poloxamer (22.56 mg) in dichloromethane, at room temperature. The resulting solution was heated at 40°C and solvent was removed until a dried blend was obtained. Cholesterol (2.75 mg) was dissolved in DMSO and then was added to the blend and dissolved at room temperature. This solution of polymers and cholesterol was added to an aqueous phase (70 mL of mQH₂O) with vigorous stirring using turrax for 2 minutes followed by stirring in a magnetic stirrer. After the formulation was prepared, nanoparticles were obtained with a mean particle size of 119±17 nm and a Zeta potential of -25±3 mV.

7. THAT Example B was carried out according to the following procedure:

Example B - Preparation of Microparticles from a Blend Without Cholesterol

Initially, a blend of polymers was prepared by dissolution of PLGA 503 (22.5 mg) and Poloxamer (22.56 mg) in dichloromethane, at room temperature. Afterwards, this solution was heated at 40°C and the solvent was removed until a dried blend was obtained. This blend was subsequently dissolved in DMSO (vortex stirring), and the solution obtained was added to an aqueous phase (70 ml. of mQH₂O) with vigorous stirring using turrax for 2 minutes followed by stirring in a magnetic stirrer. After the formulation was prepared, particles were formed (as well as some visible aggregates) with a mean particle size of 5.8±1 µm.

8. THAT Example B without cholesterol showed an inability to achieve nanoparticles, and Example A with cholesterol resulted in the production of nanoparticles, thereby evidencing that nanoparticles cannot be achieved in absence of cholesterol in the process of Grandfils, and that cholesterol is necessary in such process to produce nanoparticles.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.



ANA ISABEL VILA PENA

28. April. 2009

DATE



BALBINA FERNANDEZ MARTINEZ

28. April. 2009.

DATE